(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization

International Bureau



(43) International Publication Date 27 May 2004 (27.05.2004)

PCT

(10) International Publication Number WO 2004/044246 A3

(51) International Patent Classification7: C12O 1/68, 1/48

(21) International Application Number:

PCT/US2003/035919

(22) International Filing Date:

12 November 2003 (12.11.2003)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data: 60/425,620

12 November 2002 (12.11.2002)

(71) Applicant (for all designated States except US): ALLIED BIOTECH, INC. [US/US]; 10075 Tyler Place, #19, Ijamsville, MD 21754 (US).

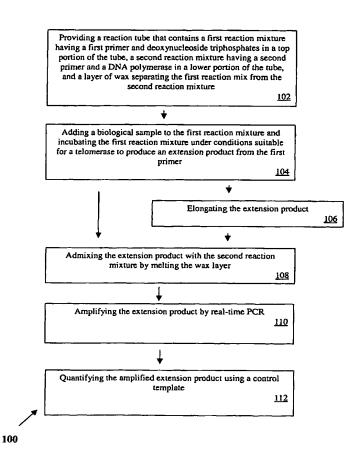
- (72) Inventors; and
- (75) Inventors/Applicants (for US only): LI, Zhuangwu

[CN/US]; 6054 Signal Flame Ct., Clarksville, MD 21029 (US). BAO, Jun [CN/US]; 16329 Sierra Ridge Way, Hacienda Heights, CA 91745 (US). MAO, Hua [CN/US]; 16329 Sierra Ridge Way, Hacienda Heights, CA 91745 (US). MA, Wenbin [CA/US]; 6502 Mallery Ct., Frederick, MD 21703 (US). LI, Lina [CN/US]; 20810 Amber Ridge Drive, Germantown, MD 20876 (US).

- (74) Agents: YE, Michael et al.; Andrews Kurth LLP, 1701 Pennsylvania Avenue, N.W. Suite 300, Washington, D.C. 20006 (US).
- (81) Designated States (national): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

[Continued on next page]

(54) Title: METHODS AND COMPOSITIONS FOR DETECTING TELOMERASE ACTIVITY



(57) Abstract: A method for determining telomerase activity using primer extension followed with real time PCR quantification is disclosed. The method of the present invention provides a rapid, sensitive and accurate measurement for telomerase activity in a biological sample. In one embodiment, the method includes the steps of: adding the biological sample to a reaction tube containing a first reaction mixture having a first primer and nucleoside triphosphates, a second reaction mixture having a second primer and a DNA polymerase, and a wax layer that separates the first reaction mixture from the second reaction mixture; incubating the biological sample with the first reaction mixture; admixing the extension product with the second reaction mixture; amplifying and quantifying the extension product using real-time PCR and a control template. In another embodiment, the detection method includes an in situ primer extension step that allows the production of the extension product within an intact cell. In this embodiment, the extension product can be preserved under appropriate conditions for an extended time before the completion of the quantification step.



(84) Designated States (regional): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

- with international search report

- before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments
- (88) Date of publication of the international search report: 21 October 2004

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.